The Royal College of Pathologists
Pathology: the science behind the cure

Guidelines on autopsy practice:

Autopsy in sickle cell disease and sickle trait

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Foreword

The autopsy guidelines published by The Royal College of Pathologists (RCPath) are bench-top guidelines for pathologists to deal with non-forensic consent and Coroners' and procurators fiscals' post-mortem examinations in a consistent manner and to a high standard. They may contain some distressing information and as such are not intended for the lay audience.

The guidelines are systematically developed statements to assist the decisions of practitioners and are based on the best available evidence at the time the document was prepared. Given that much autopsy work is single observer and one-time only in reality, it has to be recognised that there is no reviewable standard that is mandated beyond that of the FRCPath Part 2 examination or the Certificate of Higher Autopsy Training (CHAT). Nevertheless, much of this can be reviewed against ante-mortem imaging and/or other data. This guideline has been developed to cover most common circumstances. However, we recognise that guidelines cannot anticipate every pathological specimen type and clinical scenario. Occasional variation from the practice recommended in this guideline may therefore be required to report a specimen in a way that maximises benefit to the coroner and the deceased's family.

There is a general requirement from the General Medical Council (GMC) to have continuing professional development (CPD) in all practice areas and this will naturally encompass autopsy practice. Those wishing to develop expertise or specialise in pathology are encouraged to seek appropriate educational opportunities and participate in the relevant external quality assurance (EQA) scheme.

The guidelines themselves constitute the tools for implementation and dissemination of good practice.

The following stakeholders were contacted to consult for this document:

- the Sickle Cell Society
- the Human Tissue Authority (HTA).

The information used to develop this document was derived from practical experience, current medical literature and a previous version of this guideline. Much of the content of the document represents custom and practice and is based on the substantial clinical experience of the specialist expert authors. All evidence included in this guideline has been graded using modified SIGN guidance (see Appendix C). As with much of autopsy practice, the evidence level for the majority of the material in this text is Grade D. Nonetheless, many of the clinicopathological scenarios that occur in sickle cell disease (SCD) and sickle trait persons have been investigated in Coroners' courts, along with clinical expert witness contributions, and several have gone to High Court litigation for resolution on causality. Thus, the following guidelines represent current thinking and practice among the interested parties. Consensus of evidence in the guideline was achieved by review, with College members providing feedback during consultation. The sections of this autopsy guideline that indicate compliance with each of the AGREE II standards are indicated in Appendix D.

No major organisational changes or cost implications have been identified that would hinder the implementation of the guidelines. However, as sepsis has to be considered in all SCD patients who die, there must be facilities in mortuaries to undertake routine blood cultures and organ cultures, and a pathway for the material to be analysed subsequently. If these are not available, then the case should be transferred to another more appropriate mortuary. As with other uncommon and potentially complicated autopsy scenarios, such as maternal deaths, it is preferable that all SCD autopsies are performed by pathologists with an interest and suitable expertise in such matters. This does not apply to sickle cell trait (SCT), unless it is thought to directly relate to the death, the gene being frequent in the general population and sickle trait-related complications being uncommon.

A formal revision cycle for all guidelines takes place on a 5-yearly cycle. The College will ask the authors of the guideline to consider whether or not the guideline needs to be revised. A full

consultation process will be undertaken if major revisions are required. If minor revisions or changes are required, a short note of the proposed changes will be placed on the College website for 2 weeks for members' attention. If members do not object to the changes, the short notice of change will be incorporated into the guideline and the full revised version (incorporating the changes) will replace the existing version on the College website.

The guideline has been reviewed by the College's Professional Guidelines team, Death Investigation Committee, Lay Advisory Group and Specialty Advisory Committee. It was placed on the College website for consultation with the membership from 11 May 2023 to 8 June 2023. All comments received from the membership were addressed by the authors to the satisfaction of the Clinical Lead for Guideline Review.

This guideline was developed without external funding to the writing group. The College requires the authors of guidelines to provide a list of potential conflicts of interest; these are monitored by the Professional Guidelines team and are available on request. The authors of this document have declared no conflicts of interest.

1 Introduction

The sickle gene is common in the UK population. Genotypically, there are 3 main types of SCD: the HbSS, HbSC and HbS-beta thalassaemia genotypes. HbSS is the most common. Currently, the birth rate for SCD is about 1 in 2,000 births, with more than 15,000 patients living with SCD in the UK. Additionally, about 1% of all births have the HbAS genes, giving them SCT.[1–3] Demographically, these people are concentrated in and around metropolitan areas such as London, Birmingham and Manchester.

This document was created to address the needs of the autopsy pathologist/procurator fiscaldealing with deaths in persons with SCDs and SCT and indicates a technical approach and investigations that should prevent criticism of case analysis in medicolegal environments. The limitations of local coronial/procurator fiscal practice and permissions are often unique to different cases and various parts of the UK, but this documentary guidance should be satisfactory for all cases. The document is designed to be a focused bench-top guide with step-by-step examination suggestions. It highlights matters for consideration and applies to both children and adults.

The levels of evidence reflect published case reports and series, and the small number of systematic analyses of SCD and sickle trait morbid anatomy. The importance of autopsy pathology in advancing our understanding of the pathogenesis of severe SCD cannot be overstated. All our pathological knowledge of the brain, lung, cardiovascular and splenic syndromes can only come from autopsy, as these organs are not biopsied in life, and in-life imaging is often ambiguous.

1.1 Target users of this guideline

The target primary users of this guideline are consultant pathologists performing coronial, procurator fiscal and hospital/consented post-mortem examinations. The recommendations will also be of value to trainee pathologists, especially those considering Certificate of Higher Autopsy Training.

The guideline consists of the main document plus 2 new appendices. These are Appendix A – a 2-page synoptic document that conveys the essentials required to perform a sickle cell autopsy if the pathologist has no prior experience of the SCDs, and Appendix B – a brief discussion of the clinically complex cardiac syndromes that feature in modern clinical practice, where an autopsy could shed light on the cardiac pathophysiology contributing to death.

2 Role of the autopsy

Autopsy determines the pathologies that led to death and the contribution of the SCD[1,2] to death. Deaths in SCD occur in the community, in hospital after admission in crisis, in intensive care[3,4] and peri-operatively.

The gross autopsy findings are often minimal and case evaluation requires careful macroscopic examination, almost always a range of histopathological samples and usually microbiological cultures, to determine what took place. Where the sickle genotype has not been confirmed before death or autopsy findings suggest its presence, autopsy blood electrophoresis will determine the precise genotype.

SCT (HbAS genotype) is common in the UK but is rarely a significant contributor to death. However, the possibility of SCT contributing to death is relatively often raised in forensic autopsies on SCT persons, particularly when it is a death in state custody.[5,6] The autopsy and histopathology can assist in this evaluation.

[Level of evidence – GPP.]

3 Pathology encountered at the autopsy

As with all systemic diseases, having the sickle gene or disease does not necessarily mean that death resulted because of that. The main causes of death and clinical pathologies in the 3 SCD genotypes include those listed below. Some entities may be grossly evident at autopsy, but most require histopathological analysis for identification.[1,7]

The following pathologies have been broadly grouped into those that are common causes of death in sickle patients, common pathologies found in sickle patients and less common pathologies. This is based on our own experience and several case series.[7–9] Needless to say, coincidental pathologies may be present and their relative importance must be evaluated when establishing the events that led to death.

3.1 Common causes of death

- Bacterial sepsis – pneumonia, meningitis, septic shock, osteomyelitis; pneumococcal, *Haemophilus influenzae* and non-typhoid *Salmonella* infections.[1,2]
- Acute chest syndrome (ACS)[10] due to 1) pulmonary arteriolar obstruction by sickled red cells, and 2) bone marrow embolism with thrombotic microangiopathy (TMA); this often follows a sickle bone crisis with necrosis of bone marrow.
- Chronic pulmonary vascular arteriopathy, pulmonary artery hypertension and cor pulmonale.[11,12]
- Cerebrovascular accident – infarction, intracerebral haemorrhage and subarachnoid haemorrhage.[13]
- Acute hepato-splenic sequestration.[14]
- Acute and chronic renal failure – due to glomerulosclerosis, pyelonephritis and papillary necrosis.[15]
- Multi-organ failure following pan-body sickle crisis.
- Deep vein thrombosis and pulmonary thromboembolism.
- Dural venous sinus thrombosis and brain haemorrhage.
- Aplastic marrow crisis from parvovirus B19 infection (in children and less commonly in adults).

3.2 Common pathologies that are less often the direct cause of death

- Left ventricular hypertrophy and diastolic dysfunction syndromes.[16]

  Note: Cardiac disease in SCD is the subject of much clinical research. Appendix B summarises current knowledge, noting that much of the pathophysiological speculation is not necessarily supported by replicable autopsy tissue pathology.

3.3 Less common pathologies and scenarios

- Posterior reversible encephalopathy syndrome (PRES).[17]
- Ischaemic heart disease.[16]
- Pregnancy-related – with multi-organ failure and ACS, and sepsis.
- Hyperhaemolysis (post-transfusion) syndrome in adults.[18]
- Multi-organ haemosiderosis – related to therapeutic/prophylactic blood transfusions; this mainly affects heart, liver, kidney and pancreas.
- Biliary stone diseases – obstructive jaundice, cholecystitis and pancreatitis.
- Overdose of opiate painkillers: morphine/heroin, pethidine (which also causes seizures), fentanyl patches.[19]
- Gut ulceration and perforation from non-steroidal anti-inflammatory drugs (NSAIDs).
- Hydroxycarbamide – this drug is the only specific drug treatment for SCD; occasionally it damages the marrow causing pancytopaenia and associated complications.

3.4 Patients with SCT (HbAS)

- Death from acute cardio-respiratory arrest and ACS (type A – see 9.3 below) is ~30 times more frequent than among normal HbAA persons; this usually occurs after severe exertion with dehydration. Skeletal muscle rhabdomyolysis is often part of this syndrome.[5,20] At autopsy, there is grossly little or nothing to see beyond congested lungs.
- Dehydration and rapid-onset hyperosmolar diabetes can also precipitate the ACS, type A.[21]
- Dural venous sinus thrombosis is also associated with SCT and dehydration.
- A very uncommon, genetically related, cancer association with sickle trait is medullary carcinoma of the kidney.
- Some persons labelled as ‘sickle trait’ may actually have the HbSC genotype, not recognised because the laboratory tests were incomplete. If there is a mismatch between apparent sickle-related pathological features and the clinical history of trait, autopsy blood can be tested to evaluate the genotype formally.

[Level of evidence – D.]

4 Specific health and safety aspects

There are no specific health and safety aspects to consider. With current transfusion practice, HIV and hepatitis B and C are no longer inadvertently transmitted to sickle cell patients.

Sickle patients are at no more risk of HIV infection than the general ethnic population.

[Level of evidence – D.]

5 Clinical information relevant to the autopsy

- All the present relevant and past medical history details, particularly the clinical mode of death, recent operation records, drug and pain-relief therapy, current radiology.
- Laboratory results such as blood cultures, recent haematology data (haemoglobin, white blood cell count, platelets, reticulocyte count, clotting studies) and relevant biochemistry must be gathered, including the specific sickle genotype.
- Discussion with the sickle physicians is always helpful to understand the complex pathophysiological processes taking place.

[Level of evidence – D.]

6 The autopsy procedure

- In **all** cases, unless the results of recent pre-mortem blood cultures are available, take blood for culture (aerobic and anaerobic bottles) from the neck veins or heart before any incisions are made into the body.
- Full autopsy according to standard practice, with examination of the brain, and vertebral bone marrow sampling. Omitting brain examination, just because an internal organ seems to be significantly abnormal, can miss the critical intracranial pathology.
- If a long bone sickle crisis has been diagnosed clinically, it may be useful to remove 1 femur and split it longitudinally. This enables examination of marrow hyperplasia and sampling of old and recent sites of bone infarction. It can be replaced with a leg strut during reconstruction.
- Note whether there are skin ulcers on the legs.
- Photography: as in any other disease, significantly abnormal organs can be photographed to show clinical and pathology colleagues.

[Level of evidence – GPP.]

7 Specific organ systems to be considered

All organs are important; the most important in severe sickle cell morbidity and mortality are the lungs, liver and spleen, brain, heart, kidneys and bone marrow.

Specific attention is needed to the vascular and infective pathologies:

- lungs: pulmonary artery hypertension, thrombotic obstruction of arteries, thromboembolism, pneumonic inflammation, generalised congestion
- heart: the coronary arteries, left and right ventricular hypertrophy
- brain: the circle of Willis (CoW) is a critical indicator of cerebrovascular disease, new and old ischaemic strokes, intracerebral haemorrhage, subarachnoid haemorrhage, meningitis, dural venous sinus thrombosis
- liver: size, congestion, fibrosis, portal vein thrombosis
- spleen: the size (tiny remnant to massive sequestration enlargement); infarcts and fibrotic nodules

- kidneys: pyelonephritis, papillary necrosis, cortical necrosis
- bone marrow: old and new vertebral/long bone infarcts, extent of haemopoietic marrow (hyperplasia), osteomyelitis
- biliary system: bile stones, cholecystitis, pancreatitis
- pelvic and leg vein thrombosis if there is pulmonary thromboembolism.

[Level of evidence – D.]

8 Organ retention

In general, whole organ retention is not required; the exception is the brain in cases of cerebrovascular and haemorrhagic pathology.

[Level of evidence – GPP.]

9 Histological examination

Histological studies are essential to diagnose and to understand the pathogenesis of sickle-related death. Gross observation and pathological guesswork alone will fail to provide the correct cause of death within the sickle cell complex of disorders; this will not satisfy clinicians or help them with clinical governance issues and will certainly not satisfy the relatives of the deceased.

9.1 Tissue sampling

The following represents best practice for all cases; this is the recommended minimum if histology is to be sent for expert review.

Organ	**Recommended sampling**
Heart	5 blocks from a mid-horizontal slice: anterior and posterior right ventricle (RV), and the 4 quadrants of the left ventricle (LV). Epicardial coronary arteries, if stenosed.
Lungs	One sample per lobe and arterial emboli/thrombi, if present.
Spleen	As per usual protocols.
Liver	As per usual protocols.
Pancreas	Sample if it appears fibrosed/possible haemosiderosis.
Kidney	Include cortex, medulla, pyramid and calyx.
Brain	**CoW**: sample any aneurysm, if present. If not, and in all cases with cerebral or subarachnoid haemorrhage, dissect off the CoW, fix it entirely, embed in a medium-large size block and cut step sections. **Brain**: If there is cerebral haemorrhage, ischaemic stroke, meningitis or venous sinus thrombosis, sample as usual. If **PRES** was suspected in life, sample the occipital lobes.[17]
Bone	Lumbar vertebral bone in all cases. Femoral bone and marrow if it has been examined.
Other	Deep vein thrombosis, if present, with the vein wall. Recent operation sites. Septic foci not already sampled (e.g. gall bladder).

	Skeletal muscle if rhabdomyolysis is relevant, particularly in SCT persons dying following exertion.

Formalin fixation: ideally use buffered formalin, to reduce artefactual post-mortem sickling of red cells in sickle disease and sickle trait persons. Thereafter, routine processing to paraffin is appropriate.

[Level of evidence – D.]

9.2 Bacteraemic sepsis

For the evaluation of bacteraemic sepsis, the following tissue samples and special stains are essential.[22]

Tissue	Immunohistochemistry (IHC)	Pathology seen in sepsis
Spleen, liver and bone marrow	CD68 IHC	Macrophage haemophagocytosis
Lung and heart	CD54 IHC	Up-regulation of endothelial cell intercellular adhesion molecule (ICAM-1)
Lung and kidney	CD61 IHC Fibrin	Staining of fibrin and platelets to demonstrate TMA
Spleen and lymph nodes	CD68 IHC	Lymphoid atrophy; haemophagocytosis

[Level of evidence – GPP.]

9.3 Specific points on interpretation of histology

9.3.1 Lungs

- ACS has a clinical, not pathological, case definition,[1,2,14] but histopathology can support or refute the diagnosis. The broadest depiction is rapid development of respiratory signs and symptoms and a new infiltrate on chest X-ray. There are at least 2 pathogenetic and histopathological versions:[23]
  - type A: severe distension of arterioles, capillaries and venules by packed sickled red cells (i.e. a pan-lung sickle crisis); there may also be local infarction; if there is no distension by sickled red cells present, it is not the ACS
  - type B: embolism of necrotic bone marrow to small pulmonary arteries, prompting local thrombosis, intravascular sickling and acute cor pulmonale.
- Chronic sickle cell pulmonary arteriopathy is the cause of pulmonary artery hypertension and chronic cor pulmonale in SCD; it is a progressive intimal thickening of medium and small size pulmonary arteries,[24] there may be associated episodes of local thrombosis contributing to the stenosis.[25]
- Pulmonary fat embolism is always present in persons who have cardiopulmonary resuscitation (CPR) before death.[26] Distinguish this phenomenon from marrow tissue embolism which, in SCD, usually represents pre-mortem degenerate marrow necrosis; thus, it includes marrow tissue along with fat globules.
- Cardiac disease in SCD is complex. Appendix B depicts the myocardial dysfunction syndromes, where the histopathology of the right and left ventricle will contribute to their categorisation and diagnosis. A small proportion of sickle patients die with acute myocardial infarction yet have patent coronary arteries; this probably results from abnormal cardiac microcirculation and systemic hypoxia.

9.3.2 Lumbar vertebral bone

- Note the cellularity of haematopoietic lines, haemophagocytosis, zones of infarction and viral inclusion bodies (parvovirus B19).

9.3.3 Spleen[12]

- Gamna–Gandy nodules: these represent recurrent intrasplenic vascular crises with small infarctions and fibrosis. They are foci of fibrosis with iron and calcium deposition. They accumulate over decades, resulting in the tiny (5 g or less) non-functioning splenic remnant.
- Splenic sequestration: expansion of the red pulp, with aggregates of tightly packed sickled red cells.

9.3.4 Liver

- Hepatic sequestration manifests as severe congestion and expansion of the sinusoids, packed with sickle red cells.
- Haemphagocytosis by Kupffer cells.

9.3.5 CoW[11,27]

- Chronic sickle cell arteriopathy is the major cause of stroke and haemorrhage; histologically it is a combination of non-atherosclerotic intimal thickening and fibrosis, and regions of artery wall degeneration with disintegration of the elastic.[11]

[Level of evidence – D.]

10 Toxicology and other tests

- Toxicology screening in SCD patients (peripheral blood, urine, vitreous) is required in the usual circumstances of suspicion of illicit drug- or alcohol-related death.
- A drug screen is essential for sickle trait persons dying under exertion, where a positive drug test reduces the likelihood that SCT contributed to the death but does not eliminate that possibility.
- If opiates were administered during the final medical management, and there are questions over the dosage, measuring morphine is important.
- Note: fentanyl is not always detected by routine screening for drugs of abuse; it must be specified (fentanyl patches are a commonly used painkiller in sickle cell patients).
- Mast cell tryptase levels are required only if there is suspicion of acute anaphylaxis.

[Level of evidence – D.]

11 Other samples required

- Bacterial infection: blood cultures in most cases (see above); focal sepsis cultures if grossly evident.
- Spun blood for serology, e.g. B19 virus infection.

- Whole blood if the red cell sickle status had not been evaluated pre-autopsy but is suspected clinically or morbid anatomically.

[Level of evidence – D.]

12 Imaging

Imaging-based post-mortem examination should never be undertaken without an expert external examination of the body having first been performed by an appropriately trained and experienced individual. In principle, all cadavers for autopsy should have been scanned – during hospital admission or in the mortuary. However, the clinical pathology of SCD mortality is subtle and there is no role for CT or MRI scanning as an alternative to formal autopsy examination.

[Level of evidence – D.]

13 Clinicopathological summary

- Determine whether SCD is the underlying factor in the cause of death sequence, played a contributory role or was irrelevant to the cause of death.
- Consider whether drug overdose caused fatal respiratory depression or seizures.
- Lay out the pathological sequence logically to enable the treating clinicians and those close to the deceased reviewing the autopsy report to easily access the information. The report may also be reviewed for medico-legal purposes.
- In sickle trait deaths, consider whether a cardio-pulmonary collapse could have resulted from a sickle chest crisis under stress.
- Consult a more experienced pathologist to review the case and histology if the pathology and cause of death are not clear.

[Level of evidence – GPP.]

14 Examples of cause of death opinions/statements

1a. Acute cardio-respiratory failure
1b. Acute chest syndrome following painful crisis
1c. Sickle cell disease

1a. Anaemia
1b. Hepato-splenic sequestration
1c. Sickle cell disease

1a. Severe sepsis
1b. Pneumococcal bacteraemia
1c. Sickle cell disease

1a. Cardiopulmonary failure/cor pulmonale
1b. Chronic sickle pulmonary arteriopathy
1c. Sickle cell disease

1a. Subarachnoid haemorrhage

1b. Chronic sickle cerebral vasculopathy
1c. Sickle cell disease

1a. Acute cardio-respiratory failure
1b. Exertion and sickle cell trait (HbAS)

15 Criteria for audit

The following standards are suggested criteria that might be used in periodic reviews to ensure a post mortem report for coronial autopsies conducted at an institution complies with the national recommendations provided by the 2006 National Confidential Enquiry into Patient Outcome and Death (NCEPOD) study:

- Supporting documentations:
  - standards: 95% of supporting documentation was available at the time of the autopsy
  - standards: 95% of autopsy reports documented are satisfactory, good or excellent
- Reporting internal examination:
  - standards: 100% of the autopsy report must explain the description of internal appearance
  - standards: 100% of autopsy reports documented are satisfactory, good or excellent
- Reporting external examination:
  - standards: 100% of the autopsy report must explain the description of external appearance
  - standards: 100% of autopsy reports documented are satisfactory, good or excellent.

A template for coronial autopsy audit can be found on The Royal College of Pathologists' website.

16 References

Note: there is relatively little published on the modern pathology and morbid anatomy of sickle cell disease. The following are useful clinical and pathogenetic reviews, with some autopsy case reports and series.

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Appendix A Synoptic sickle cell disease autopsy guidelines

Note: This version is intended for pathologists with little or no prior experience of SCD. If this protocol is followed and all relevant samples taken, the true disease chronology and diagnosis should be achievable, with reference to the full sickle autopsy document and expert consultation advice.

Patients with known SCT (HbAS) do **not** require the full SCD autopsy protocol, **except** in circumstances of sudden death under unusual conditions of exertion, heat-related disease or state detention.

A full autopsy should be performed according to standard practice. The head and brain should always be examined in all cases.

Prior to autopsy

- Ascertain circumstances of death with reference to coroner referral, GP notes, hospital medical records, including laboratory and imaging results.
- Where possible, establish SCD genotype, previous and current treatments, and whether any known complications of SCD.

External examination

- Height and weight, BMI, medical devices, transdermal medication patches, scars, jaundice, conjunctival pallor, joint swelling, pitting oedema, cutaneous ulcers, renal dialysis lines.

Gross internal examination

Weigh the heart, lungs, kidneys, liver, spleen and brain:

- heart
  - calculate ratio of heart to body weight. Measure left ventricular (LV) wall thickness (increased secondary to chronic anaemia) and right ventricular (RV) wall thickness (increased in pulmonary hypertension). Diastolic dysfunction is commoner than systolic dysfunction in SCD and is often associated with increased LV mass
  - assess for evidence of chamber dilation, myocardial infarction, often in absence of significant coronary atherosclerosis, diffuse myocardial fibrosis (due to microvascular disease)
- lungs
  - assess for pulmonary oedema, infection/consolidation, infarction, thromboembolism, pulmonary artery prominence and arteriosclerosis, interstitial fibrosis
- gastrointestinal system
  - assess for peptic ulcer disease and mesenteric ischaemia
- hepatopancreatobiliary system
  - assess liver for hepatomegaly, cirrhosis/fibrosis, infarction, abscess, hepatic or portal vein thrombosis
  - assess pancreaticobiliary system for cholestasis, cholelithiasis (usually black pigment stones), acute and chronic cholecystitis, ascending cholangitis, pancreatitis, pancreatic fibrosis
- renal and genitourinary system
  - assess for scarring, atrophy, renal papillary necrosis, pyelonephritis and malignancy (e.g. medullary carcinoma in SCT)

- central nervous system
  - assess for stroke (ischaemic stroke in patients <20 years or >30 years, intracranial haemorrhage in intermediate age group), subarachnoid haemorrhage, subdural and extradural haematomas, CoW cerebral vasculopathy (stenosis and/or attenuation of large intracranial arteries), venous sinus thrombosis, cerebral infection (meningitis and cerebral abscesses)
- haematolymphoid system
  - assess for autosplenectomy, splenomegaly, splenic infarction
- musculoskeletal systems
  - assess for avascular necrosis, osteomyelitis – where clinically indicated; always open a lumbar vertebra.

Histology (essential)

- Heart
  - take 6 samples from a mid-horizontal slice: anterior and posterior RV (in 1 block), and the 4 quadrants of the LV
  - microscopically, assess for hypertrophy, infarction, patchy or diffuse interstitial fibrosis, and siderosis.
- Lungs
  - one representative section from each lobe of both lungs, in addition to other areas of abnormality
  - microscopically, assess pulmonary arteries and arterioles for thickening and pulmonary artery hypertension, recanalisation (previous venous thromboembolism [VTE]), fat and bone marrow embolism, in-situ thrombosis, and interstitial fibrosis
  - ACS: histological features include severe distension of arterioles, capillaries and venules by packed sickled red cells, with or without local infarction, in ‘type A’ ACS; embolism of necrotic bone marrow to small pulmonary arteries, prompting local thrombosis and intravascular sickling in ‘type B’ ACS.
- Liver
  - assess for hepatic histological findings of SCD including Kupffer cell hyperplasia with erythrophagocytosis, sinusoidal dilatation, and intrasinusoidal sickled erythrocytes. Other features include haemosiderosis, focal nodular hyperplasia, infarction and extramedullary haematopoiesis. Acute conditions including acute sickle hepatic sequestration crisis
  - assess for biliary manifestations including sickle cholangiopathy (obstruction, cholestasis, biliary fibrosis), cholecystitis, ascending cholangitis
  - assess for features of viral hepatitis – acute and chronic, and fibrosis.
- Pancreas
  - sample if it appears fibrosed or haemosiderotic.
- Renal and genitourinary system
  - take a section from each kidney to include cortex, medulla, pyramid and calyx
  - in cortex, assess for focal infarction and scarring. Assess glomeruli for hypertrophy, mesangial proliferation, structural abnormalities, most commonly focal segmental glomerulosclerosis (FSGS), and less frequently membranoproliferative glomerulonephritis-like (MPGN-like) disease, and TMA. Assess tubule-interstitium for tubular epithelial haemosiderin deposition, tubular atrophy, myoglobin casts, fibrosis

  - assess medulla for congestion of capillaries, necrosis and fibrosis. Assess for papillary necrosis.

- Central nervous system
  - sample any aneurysm from the CoW, if present. If not, and in all cases with cerebral or subarachnoid haemorrhage, dissect off the CoW, fix it entirely, and embed in a medium-large size block
  - sample brain as per usual protocol if there is cerebral haemorrhage, ischaemic stroke, meningitis or venous sinus thrombosis
  - if PRES was suspected in life, sample the occipital lobes
  - histologically, assess for hypoxic neurone damage, infarction, fat embolism
  - CoW – chronic sickle arteriopathy (combination of non-atherosclerotic intimal thickening, medial fibrosis, and artery wall degeneration with disintegration of the elastic), which may not be seen macroscopically.
- Haematolymphoid and musculoskeletal systems
  - sample spleen if identified. Assess for disordered white pulp architecture and Gamna-Gandy bodies (sclerosiderotic nodules with iron and calcium deposition). Assess for acute splenic sequestration and infarction
  - sample lumbar vertebral bone marrow in all cases. Sample femoral bone and marrow if opened. Assess for cytopenia, infarction, necrosis, hyperplasia (commonly erythroid) and viral inclusion bodies (parvovirus B19).
- Other organs as indicated by scenario of death: deep vein thrombosis with vein wall; skeletal muscle if rhabdomyolysis suspected.
- Useful ancillary tests
  - elastic staining (pulmonary hypertension), Perls stain (haemosiderosis), Gram stain.

Peripheral blood

- Blood cultures: aspirate aerobic and (if possible) anaerobic cultures from jugular veins or directly from heart
- Serology as required, e.g. for parvovirus B19 or viral hepatitis
- Toxicology: if illicit drug- or alcohol-related death suspected; many SCD patients take large doses of prescribed morphine and other opioids
- Un-preserved blood – haemoglobin electrophoresis for sickle genotype, if not known

Vitreous humour

- For toxicology, if illicit drug- or alcohol-related death suspected.

Urine

- For toxicology, if illicit drug- or alcohol-related death suspected; pneumococcal antigen test if suspected.

Appendix B Cardiac complications in sickle cell disease

Researched and drafted by Dr Naoimh Herlihy.

Cardiovascular causes are reported to account for up to 32% of deaths in SCD.[28] Several autopsy series have reported cardiac pathologies including heart failure, myocardial ischaemia and infarction, sudden cardiac death and cardiac arrhythmias as causes of death in SCD.[28–34] Common premorbid cardiovascular conditions reported in SCD patients at autopsy include pulmonary hypertension, systemic hypertension, myocardial infarction and arrhythmias including atrial fibrillation, supraventricular tachycardia and nonfatal ventricular fibrillation.[32]

Note: Our London sickle experience (>100 autopsies) finds proportionately less severe cardiac disease than indicated in the literature; it is likely that there is a reporting bias involved in publications. Nearly all the heart function abnormalities are described in patients from non-invasive and invasive in-life investigations, with little or no pathology depicted.

Heart weight (including ratio of heart to body weight), left ventricular (LV) wall thickness and right ventricular (RV) wall thickness are important to assess at autopsy in SCD.

Dilated cardiomyopathy

Although unvalidated, there is growing evidence to support the development of a dilated cardiomyopathy with preserved ejection fraction in patients with SCD[34,35]. More commonly, dilatation occurs from volume overload, secondary to anaemia.

Chronic anaemia in SCD causes a hyperdynamic state, in which there is increased cardiac output and volume overload secondary to peripheral vascular dilation and renin-angiotensin-aldosterone system activation.[36] High left ventricular volumes result in dilation of the left ventricle.[35] Increased wall stress then results in compensatory cardiac remodelling in the form of eccentric myocardial hypertrophy, with thickening of the left ventricular wall and elongated myofibres.[33,35,37]

Four-chamber dilatation has been demonstrated in living patients with SCD.[34] Cardiomegaly is seen in SCD patients at autopsy, with increased left ventricular wall thickness.[33,38–41] Right ventricular hypertrophy may also be seen, often in the setting of pulmonary hypertension,[31] where it may be accompanied by other microscopic features of pulmonary arterial hypertension, such as localised plaque formation in the walls of the pulmonary artery.[38]

It may not be possible to separate out right ventricular changes in pulmonary artery hypertension from those due to left sided heart disease. Left ventricular hypertrophy may be seen with or without right ventricular systolic dysfunction/cor pulmonale.

Diastolic dysfunction

Left ventricular diastolic dysfunction is common in SCD, occurring in around a quarter of patients,[33] and is an independent predictor of early mortality in adults.[42,43] The mechanism is unknown,[44] but is likely multifactorial, with possible causes including systemic vasculopathy affecting afterload,[34] the aforementioned anaemia-related compensatory myocardial hypertrophy and LV dilatation, remodelling due to recurrent microvascular ischaemic events, diffuse myocardial fibrosis[45] or, rarely in SCD, cardiac iron deposition.[35,46,47]

Diastolic dysfunction can only be diagnosed by functional studies in living patients. Although transthoracic echocardiogram studies have linked diastolic dysfunction to increased LV mass,[35] a study utilising more accurate cardiac magnetic resonance (CMR) did not confirm this.[34] Left ventricular systolic function is usually preserved in SCD.[33,35,37]

Diffuse myocardial fibrosis

Diffuse myocardial fibrosis has been reported in both imaging[34,45] and autopsy studies[30] in SCD. Desai et al[34] reported 3 different histological patterns: a low-grade diffuse myocardial fibrosis pattern, severe and transmural fibrosis pattern, and a mild and patchy pattern. The pathophysiology of myocardial fibrosis in SCD is unknown but may be due to anaemia, repeated vaso-occlusive episodes, inflammation and ischaemia, and may contribute to diastolic dysfunction.[36,45]

Unique sickle cell cardiomyopathy?

A paradigm for a unique SCD-related cardiomyopathy has been recently proposed in SCD, with combined restrictive physiology (characterised by diastolic dysfunction, left atrial enlargement and typically normal systolic function) and hyperdynamic physiology (characterised by left ventricular dilatation and eccentric left ventricular hypertrophy, due to chronic anaemia with increased cardiac output and volume overload),[44,48,49] although prospective studies are lacking, as is relevant human tissue pathology.

Pulmonary hypertension

Pulmonary hypertension (PH) is defined as a mean pulmonary artery pressure of >20mmHg at rest as measured by right heart catheterisation. SCD patients with PH may have pulmonary arterial hypertension (precapillary), pulmonary venous hypertension (postcapillary) or a combination of both based on right heart catheterisation.[35] PH in SCD can be due to increased pulmonary vascular resistance and therefore increased pulmonary arterial pressure due to left ventricular hypertrophy, diastolic dysfunction, haemolysis- and hypoxia-associated endothelial dysfunction, smooth muscle cell and intimal proliferation, and in-situ thrombosis.[37]

As patients with SCD may have multifactorial mechanisms of increased pulmonary pressures including elements of WHO Groups 1 to 4, PH in SCD is currently classified as WHO Group 5 (PH due to unclear multifactorial mechanisms). In autopsies in the setting of PH, pulmonary arterial and venous thickening is seen histologically,[14,23] as are microthrombotic and thromboembolic lesions.[30,31,39,41] PH in SCD is independently associated with mortality.[51] Right heart enlargement and right heart failure may result from PH.[37] Right ventricular systolic dysfunction (cor pulmonale) is a common cause of death in SCD patients with pulmonary hypertension[46] and can develop acutely.[35]

Myocardial ischaemia

Myocardial infarction has been reported in 9.7–20% of autopsies on SCD patients,[39] usually in the absence of traditional cardiac risk factors with no gross major vessel obstruction or significant atherosclerosis of the major coronary arteries.[38] Possible contributory factors to myocardial ischaemia in SCD include anaemia, vasospasm, procoagulant state and acute and chronic microvascular occlusion in vaso-occlusive sickling events and systemic fat emboli syndrome.[35,47]

Arrhythmias

Prolonged corrected QT interval (QTc), supraventricular and ventricular dysrhythmias and sudden death are reportedly common in SCD.[29,46] Arrhythmias occur in nearly 6% of SCD-related admissions, are associated with a higher risk of all-cause inpatient mortality[52] and have been reported as the cause of death in 14% of adults with SCD.[32] Prolonged QTc has been demonstrated as an independent risk factor for increased mortality in SCD.[53] The risk of serious arrhythmia is higher during vaso-occlusive crisis.[54]

Appendix C Summary table – explanation of grades of evidence

(modified from Palmer K *et al. BMJ* 2008;337:1832)

Grade (level) of evidence	Nature of evidence
Grade A	At least 1 high-quality meta-analysis, systematic review of randomised controlled trials or a randomised controlled trial with a very low risk of bias and directly attributable to the target cancer type or A body of evidence demonstrating consistency of results and comprising mainly well-conducted meta-analyses, systematic reviews of randomised controlled trials or randomised controlled trials with a low risk of bias, directly applicable to the target cancer type.
Grade B	A body of evidence demonstrating consistency of results and comprising mainly high-quality systematic reviews of case-control or cohort studies and high-quality case-control or cohort studies with a very low risk of confounding or bias and a high probability that the relation is causal and which are directly applicable to the target cancer type or Extrapolation evidence from studies described in A.
Grade C	A body of evidence demonstrating consistency of results and including well-conducted case-control or cohort studies and high-quality case-control or cohort studies with a low risk of confounding or bias and a moderate probability that the relation is causal and which are directly applicable to the target cancer type or Extrapolation evidence from studies described in B.
Grade D	Non-analytic studies such as case reports, case series or expert opinion or Extrapolation evidence from studies described in C.
Good practice point (GPP)	Recommended best practice based on the clinical experience of the authors of the writing group.

Appendix D AGREE II compliance monitoring sheet

The guidelines of The Royal College of Pathologists comply with the AGREE II standards for good quality clinical guidelines. The sections of this guideline that indicate compliance with each of the AGREE II standards are indicated in the table below.

AGREE II standard	Section of guideline
Scope and purpose	
1 The overall objective(s) of the guideline is (are) specifically described	Foreword
2 The health question(s) covered by the guideline is (are) specifically described	Foreword, 1
3 The population (patients, public, etc.) to whom the guideline is meant to apply is specifically described	Foreword, 1
Stakeholder involvement	
4 The guideline development group includes individuals from all the relevant professional groups	Foreword
5 The views and preferences of the target population (patients, public, etc.) have been sought	Foreword
6 The target users of the guideline are clearly defined	1
Rigour of development	
7 Systematic methods were used to search for evidence	Foreword
8 The criteria for selecting the evidence are clearly described	Foreword
9 The strengths and limitations of the body of evidence are clearly described	Foreword
10 The methods for formulating the recommendations are clearly described	Foreword
11 The health benefits, side effects and risks have been considered in formulating the recommendations	n/a
12 There is an explicit link between the recommendations and the supporting evidence	Throughout
13 The guideline has been externally reviewed by experts prior to its publication	Foreword
14 A procedure for updating the guideline is provided	Foreword
Clarity of presentation	
15 The recommendations are specific and unambiguous	Throughout
16 The different options for management of the condition or health issue are clearly presented	Foreword
17 Key recommendations are easily identifiable	3–14
Applicability	
18 The guideline describes facilitators and barriers to its application	Foreword
19 The guideline provides advice and/or tools on how the recommendations can be put into practice	Foreword
20 The potential resource implications of applying the recommendations have been considered	Foreword
21 The guideline presents monitoring and/or auditing criteria	15
Editorial independence	
22 The views of the funding body have not influenced the content of the guideline	Foreword
23 Competing interest of guideline development group members have been recorded and addressed	Foreword